*REMARKS*

**Amendments**

Claims 1 and 11 have been amended to clarify that upon expression the VA1 RNA contains the RNAi molecule which is processed from the VA1 RNA to become a substrate for Dicer. Support for this amendment can be found in, for example, Figure 3 and its description in paragraph [0024] of the published application.

Claim 2 has been amended to specify that the non-essential stem region is in the coding sequence for the VA1 RNA. Support for this amendment can be found in, for example, Figure 2.

It is submitted that these amendments do not constitute new matter, and their entry is requested.

**Claim Objection**

The Examiner has objected to claims 2 and 3 under 37 C.F.R. 1.75(c) for failing to further limit the subject matter of claim 1. Applicants submit that the amendment to claim 2 obviates this objection. Withdrawal of this objection is requested.

**Summary of the Invention**

In one aspect, the present invention is directed to an expression cassette which comprises an adenoviral VA1 gene and a nucleic acid encoding an interfering RNA (RNAi) molecule. The adenoviral VA1 gene comprises the adenoviral VA1 promoter and a coding sequence for the VA1 RNA. The nucleic acid is inserted within the adenoviral VA1 coding sequence. The nucleic acid encodes a hairpin siRNA (shRNA) or a precursor microRNA (precursor miRNA). The RNAi molecule is a substrate for Dicer (i) upon expression of the VA1 RNA which includes the RNAi molecule and (ii) after the RNAi molecule is processed out of the VA1 RNA. That is, in order to be a substrate for Dicer, the RNAi molecule must be removed from the VA1 RNA. As shown in Figure 3, the RNAi molecule is cleaved from the VA1 RNA (processed intermediate) and this processed intermediate is then a substrate for Dicer which produces the active siRNA or miRNA

molecule. Hybridization of the active siRNA molecule to the mRNA in the cell leads to cleavage of the mRNA.

In a second aspect, the present invention is directed to a mammalian cell into which the above-defined expression cassette has been introduced.

**Rejection Under 35 U.S.C. § 103(a)**

The Examiner has rejected claims 1, 2 and 11-16 under 35 U.S.C. § 103(a) as being obvious over Agami et al. (US 7,241,618) taken with Doglio et al. (US 5,837,503) in further view of either Yu et al. (*Proc Natl Acad Sci USA* **99**:6047-6052, 2002) or Ambros (*Cell* **107**:823-826, 2001). Applicants traverse this rejection.

The Examiner cites Agami et al. for its disclosure of an expression cassette comprising an adenoviral VA1 promoter operably linked to an siRNA molecule which could be an shRNA molecule. The Examiner further cites Agami et al. for its disclosure that the siRNA molecule is a substrate for Dicer. The Examiner notes that Agami et al. does not teach a precursor miRNA and does not teach the claimed structural limitations of the VA1 promoter.

The Examiner cites Doglio et al. for its disclosure of an expression cassette in which a nucleic acid encoding either an antisense oligonucleotide or a ribozyme has been inserted between or outside the boxes A and B constituting the promoter of the VA1 gene or into the VA1 gene.

The Examiner cites Yu et al. for its disclosure of an RNA pol III vector comprising shRNA which can inhibit expression in mammalian cells and cites Ambros for its disclosure of miRNA.

The Examiner then concludes that it would have been *prima facie* obvious to combine the teachings of these references to produce an expression cassette comprising an adenoviral VA1 promoter wherein an RNAi molecule is contained within a non-essential stem region of the promoter or coding region of the VA1 gene. He contends that a skilled artisan would have been motivated to combine these teachings to avoid reducing the activity of the promoter or to successfully express the RNAi molecule in cells.

With respect to Applicants' prior argument concerning no motivation to combine the references in the manner set forth in the previous rejection, the Examiner contends that the skilled artisan would have been motivated to combine the prior art to successfully express an RNAi molecule in a cell, apparently because the skilled artisan understands that RNA transcripts are cleaved citing to columns 1-3 of Agami et al. and to Sharp et al. (*Genes & Development* **15**:485-490, 2001). The Examiner contends that the transcript would be cleaved and become a substrate for Dicer when expressed in cells as taught by Agami et al. and Sharp et al.

With respect to Applicants' prior argument that Rossi et al. (US 6,100,087) teaches away from the present claimed subject matter because the antisense or ribozyme coding sequences inserted into the VA1 coding sequence are not processed out of the VA1 transcript and that it is necessary for the RNAi molecule to be cleaved out of the VA1 RNA in order to become a substrate for Dicer, the Examiner makes the same comments as with Applicants' argument concerning lack of motivation.

It appears to Applicants that the Examiner is confusing the effect of RNAi, i.e., that siRNA or shRNA or miRNA hybridize to RNA transcripts in the cell which are then cleaved. The portion of Agami et al. and Sharp et al. cited by the Examiner merely describe this feature of RNAi. This cited art, at best, only shows that dsRNA molecules are a substrate for Dicer to produce smaller RNA molecules which then function to lead to the cleavage of RNA transcripts. There is no teaching or suggestion in Agami et al. or Sharp et al. that the VA1 transcript is cleaved by Dicer in mammalian cells. In the absence of any such teaching in these references, the Examiner's assertions that a skilled artisan would expect that the RNAi molecules inserted into the VA1 transcript would be cleaved out to become a substrate for Dicer is incorrect, especially in view of the teachings of Doglio et al., Rossi et al. ('087) and Cagnon et al. (*Antisense Nucl Acid Drug Dev* **10**:251-261, 2000) as discussed in further detail below. Applicants are not arguing that the shRNA or precursor miRNA molecules are not known to be substrates of Dicer when they exit in the cell as such molecules. However, Applicants submit that these molecules are not known to be substrates of Dicer when they are incorporated into the VA1 transcript, i.e., VA1 RNA. If these RNAi molecules are not processed

out of the VA1 transcript, they are not a substrate for Dicer. There is no teaching in the prior art that any molecules inserted into the VA1 transcript are processed out of the transcript.

In fact the prior art teaches exactly the opposite. Specifically, Doglio et al. teaches that the antisense oligonucleotide inserted into the VA1 transcript is functional because the antisense oligonucleotide appears "on the secondary structure as 'extrusions' relative to the principal axis of the VA RNA." Because of this structure, the antisense oligonucleotides "are accessible to sense RNAs." See column 4, lines 54-60 of Doglio et al. The ribozyme sequences are functional in the same manner as the antisense oligonucleotides. See column 5, lines 26-28 of Doglio et al. Thus, Doglio et al. teaches that the RNAi molecules inserted into the VA1 transcript, i.e., antisense oligonucleotides and ribozymes are not processed from the VA1 transcript. This teaching of Doglio et al. is confirmed by Rossi et al. ('087) and Cagnon et al. That is, Rossi et al. ('087) and Cagnon et al. teach that it was known that the ribozyme in the VA1 transcript was not processed out of the VA1 transcript. See, e.g., column 5, lines 28-33 of Rossi et al. ('087) which teaches that the ribozyme is active without being removed from the VA1 transcript. Thus, Applicants submit that the prior art clearly teaches that the RNAi molecules of antisense oligonucleotides and ribozymes are not processed out of the VA1 transcript into which they have been inserted by being expressed from an expression construct comprising the adenoviral VA1 gene and a nucleic acid encoding the antisense oligonucleotide or the ribozyme.

Since the prior art (Doglio et al., Rossi et al. ('087) and Cagnon et al.) teach that the antisense oligonucleotide RNAi molecule or the ribozyme RNAi molecule is not cleaved out of the VA1 transcript, Applicants submit that there is no motivation to substitute a different interfering molecule, e.g., shRNA or precursor miRNA which must be processed out of the VA1 transcript, for the antisense oligonucleotide or ribozyme of Doglio et al. which, as the art shows, is not processed out of the VA1 transcript. It is only upon removal of the shRNA or precursor miRNA from the VA1 transcript that it becomes a substrate for Dicer to produce the active siRNA or miRNA.

Furthermore, because Doglio et al., Rossi et al. ('087) and Cagnon et al. specifically teach that the antisense oligonucleotide RNAi molecule or ribozyme RNAi molecule are not processed out

of the VA1 transcript, there is no reasonable expectation of success that any other RNAi molecule inserted into the VA1 transcript would be processed out of the VA1 transcript so that it could then function as a substrate for Dicer to produce the active molecule which hybridizes to the target mRNA. Since there is no reasonable expectation in the art that the shRNA molecule or precursor miRNA molecule would be processed out of the VA1 transcript to become a substrate for Dicer, Applicants submit that the claimed invention does more than yield predictable results.

The above arguments are substantiated by the Rule 132 Declaration of Dr. John Rossi submitted concurrently herewith. Dr. Rossi discusses the teachings of Agami et al. and Sharp et al. concerning transcript cleavage in Paragraphs 12-14. Dr. Rossi states that Agami et al. and Sharp et al. teach that dsRNAs added to cells is cleaved by Dicer to form 21-25 (Agami et al.) or 21-23 (Sharp et al.) nucleotide dsRNAs. Since the dsRNAs are added to the cells, the dsRNAs are accessible to Dicer for cleavage to the smaller dsRNAs. Dr. Rossi also states that Agami et al. and Sharp et al. teach that the complementary strand of these smaller dsRNAs then hybridizes to a target mRNA transcript, and it is the hybridization of the complementary strand to the target mRNA transcript that leads to the cleavage of the target mRNA transcript. The cleavage of the target mRNA transcript only occurs if there is an active siRNA in the cell, e.g., an siRNA cleaved from a longer dsRNA by Dicer. Dr. Rossi further states that there is no teaching in either Agami et al. or Sharp et al. that the adenoviral VA1 RNA transcript is cleaved in a cell or that the adenoviral VA1 RNA transcript is a substrate for Dicer. Thus, Applicants submit that a skilled artisan does not understand that the adenoviral VA1 RNA transcript is cleaved in contrast to the contention made by the Examiner.

Dr. Rossi then discusses the teachings of the prior art, including prior art cited by the Examiner, concerning cleavage of the adenoviral VA1 RNA transcript in Paragraphs 15-18. Dr. Rossi states that Doglio et al. (cited by the Examiner), Rossi et al. (US 6,100,087) and Cagnon et al. (cited by the Examiner) all teach that an adenoviral VA1 RNA transcript containing an inhibitory RNA, i.e., an antisense oligonucleotide and/or a ribozyme, is not cleaved in a cell. Dr. Rossi states that the inhibitory RNA for each of these references is positioned in the adenoviral VA1 RNA

transcript so that it is accessible to the target mRNA transcript. Dr. Rossi also states that each of these references teaches that the inhibitory RNA is active within the adenoviral VA1 RNA transcript and does not need to be cleaved from the adenoviral VA1 RNA transcript in order to be active. Dr. Rossi further states that figures in each of these references shows that the adenoviral VA1 RNA transcript is not cleaved in a cell. Thus, Dr. Rossi states that each of these references teaches that the adenoviral VA1 RNA transcript containing an inhibitory RNA molecule is not cleaved in a cell and that the inhibitory RNA is not *per se* produced other than as a part of the adenoviral VA1 RNA transcript. Since the adenoviral VA1 RNA transcript containing an inhibitory RNA molecule is not cleaved in a cell, it is not a substrate for Dicer nor is the inhibitory RNA molecule a substrate for Dicer.

Dr. Rossi then discusses the import of the teachings of Agami et al., Sharp et al., Doglio et al., Rossi et al. ('087) and Cagnon et al. in Paragraphs 19-21. In Paragraph 19, Dr. Rossi summarizes the teachings of Agami et al. and Sharp et al. and also notes a similar teaching for microRNA in the cited Ambros reference. After summarizing these references, Dr. Rossi states that it is his opinion that a skilled artisan understands that RNA transcripts are not randomly cleaved without the appropriate cellular mechanisms. Such mechanisms include an active siRNA or miRNA molecule. In Paragraph 20, Dr. Rossi summaries the teachings of Doglio et al., Rossi et al. ('087) and Cagnon et al. After summarizing these references, Dr. Rossi states that it is his opinion that a skilled artisan understands that the VA1 RNA transcripts containing an inhibitory RNA molecule is not cleaved in a cell. Because of this understanding by the skilled artisan, Dr. Rossi further states that it is his opinion that these references teach away from the present invention. In Paragraph 21, Dr. Rossi states that the requirement that the RNAi molecule, i.e., shRNA or precursor miRNA, has to be accessible to Dicer in order to be a substrate for Dicer. Dr. Rossi also states that the art teaches that the adenoviral VA1 RNA transcript is not cleaved in a cell. Because the adenoviral VA1 RNA transcript is not cleaved and since the dsRNA must be accessible to Dicer to be a Dicer substrate, Dr. Rossi states that it is his opinion the skilled artisan would not have been motivated to combine the Agami et al. and Doglio et al. references or to combine Agami et al. and Doglio et al. with any of the

other cited references. Dr. Rossi further states that it is his opinion that there was no reasonable expectation of success in view of the specific teachings of Doglio et al., Rossi et al. ('087) and Cagnon et al. that the RNAi molecule of the present invention would be processed from the adenoviral VA1 RNA transcript containing it so that the RNAi molecule would become a substrate for Dicer. Because there is no reasonable expectation of success, Dr. Rossi states that it is his opinion that the skilled artisan would not have been motivated to make any of the proposed combinations of prior art. These opinions based on the teachings of the prior art are summarized by Dr. Rossi in Paragraph 22.

In view of the above amendments and remarks, Applicants submit that the claimed subject matter is not rendered obvious by the combination of Agami et al., Doglio et al. and either Yu et al. or Ambros. Withdrawal of this rejection is requested.

**Rejection Under 35 U.S.C. § 103(a)**

The Examiner has rejected claims 1, 2 and 3 under 35 U.S.C. § 103(a) as being obvious over Agami et al. taken with Doglio et al. in further view of either Yu et al. or Ambros and in further view of Cagnon et al. (*Antisense Nucl Acid Drug Dev* **10**:251-261, 2000). The Examiner cites Agami et al., Doglio, Yu et al. and Ambros as in the previous rejection. The Examiner cites Cagnon et al. for its disclosure of inserting an RNAi molecule (i.e., ribozyme) using a filled in NotI site that was ligated into the BstEII cleaved, filled in vector. The Examiner then concludes that it would have been *prima facie* obvious to produce an expression cassette in which the non-essential region contains a BstEII site. Applicants traverse this rejection.

As described above with respect to the previous rejection, Applicants submit that there is no teaching or suggestion in Agami et al. or Sharp et al. that the VA1 transcript is cleaved by Dicer in mammalian cells. In the absence of any such teaching in these references, the Examiner's assertions that a skilled artisan would expect that the RNAi molecules inserted into the VA1 transcript would be cleaved out to become a substrate for Dicer is incorrect, especially in view of the teachings of Doglio et al., Rossi et al. ('087) and Cagnon et al. as discussed above. As previously discussed,

Applicants are not arguing that the shRNA or precursor miRNA molecules are not known to be substrates of Dicer when they exit in the cell as such molecules. However, Applicants submit that these molecules are not known to be substrates of Dicer when they are incorporated into the VA1 transcript, i.e., VA1 RNA. If these RNAi molecules are not processed out of the VA1 transcript, they are not a substrate for Dicer. There is no teaching in the prior art that any molecules inserted into the VA1 transcript are processed out of the transcript.

In fact as discussed above, the prior art teaches exactly the opposite. Specifically, Doglio et al. teaches that the antisense oligonucleotide inserted into the VA1 transcript is functional because the antisense oligonucleotide appears "on the secondary structure as 'extrusions' relative to the principal axis of the VA RNA." Because of this structure, the antisense oligonucleotides "are accessible to sense RNAs." See column 4, lines 54-60 of Doglio et al. The ribozyme sequences are functional in the same manner as the antisense oligonucleotides. See column 5, lines 26-28 of Doglio et al. Thus, Doglio et al. teaches that the RNAi molecules inserted into the VA1 transcript, i.e., antisense oligonucleotides and ribozymes are not processed from the VA1 transcript. This teaching of Doglio et al. is confirmed by Rossi et al. ('087) and Cagnon et al. That is, Rossi et al. ('087) and Cagnon et al. teach that it was known that the ribozyme in the VA1 transcript was not processed out of the VA1 transcript. See, e.g., column 5, lines 28-33 of Rossi et al. ('087) which teaches that the ribozyme is active without being removed from the VA1 transcript. Thus, Applicants submit that the prior art clearly teaches that the RNAi molecules of antisense oligonucleotides and ribozymes are not processed out of the VA1 transcript into which they have been inserted by being expressed from an expression construct comprising the adenoviral VA1 gene and a nucleic acid encoding the antisense oligonucleotide or the ribozyme.

Since the prior art (Doglio et al., Rossi et al. ('087) and Cagnon et al.) teach that the antisense oligonucleotide RNAi molecule or the ribozyme RNAi molecule is not cleaved out of the VA1 transcript, Applicants submit that there is no motivation to substitute a different interfering molecule, e.g., shRNA or precursor miRNA which must be processed out of the VA1 transcript, for the antisense oligonucleotide or ribozyme of Doglio et al. which, as the art shows, is not processed

out of the VA1 transcript. It is only upon removal of the shRNA or precursor miRNA from the VA1 transcript that it becomes a substrate for Dicer to produce the active siRNA or miRNA.

Furthermore, because Doglio et al., Rossi et al. ('087) and Cagnon et al. specifically teach that the antisense oligonucleotide RNAi molecule or ribozyme RNAi molecule are not processed out of the transcript, there is no reasonable expectation of success that any other RNAi molecule inserted into the VA1 transcript would be processed out of the VA1 transcript so that it could then function as a substrate for Dicer to produce the active molecule which would hybridize to the target mRNA. Since there is no reasonable expectation in the art that the shRNA molecule or precursor miRNA molecule would be processed out of the VA1 transcript to become a substrate for Dicer, Applicants submit that the claimed invention does more than yield predictable results.

These points are further substantiated by the Rule 132 Declaration of Dr. John Rossi as discussed in detail above.

In view of the above amendments and remarks, Applicants submit that the claimed subject matter is not rendered obvious by the combination of Agami et al., Doglio et al., either Yu et al. or Ambros and Cagnon et al. Withdrawal of this rejection is requested.

**Rejection Under 35 U.S.C. § 103(a)**

The Examiner has rejected claims 1, 5 and 6 under 35 U.S.C. § 103(a) as being obvious over Agami et al. taken with Doglio et al. in further view of either Yu et al. or Ambros and in further view of Lorens (US 2004/0005593). The Examiner cites Agami et al., Doglio, Yu et al. and Ambros as in the previous rejection. The Examiner cites Lorens for its disclosure of an RNAi molecule having a loop containing at least 6 nucleotides. The Examiner then concludes that it would have been *prima facie* obvious to produce an expression cassette comprising an adenoviral VA promoter in which an RNAi molecule comprises a loop containing about 8 nucleotides. Applicants traverse this rejection.

As described above with respect to the previous rejection, Applicants submit that there is no teaching or suggestion in Agami et al. or Sharp et al. that the VA1 transcript is cleaved by Dicer in

mammalian cells. In the absence of any such teaching in these references, the Examiner's assertions that a skilled artisan would expect that the RNAi molecules inserted into the VA1 transcript would be cleaved out to become a substrate for Dicer is incorrect, especially in view of the teachings of Doglio et al., Rossi et al. ('087) and Cagnon et al. as discussed above. As previously discussed, Applicants are not arguing that the shRNA or precursor miRNA molecules are not known to be substrates of Dicer when they exit in the cell as such molecules. However, Applicants submit that these molecules are not known to be substrates of Dicer when they are incorporated into the VA1 transcript, i.e., VA1 RNA. If these RNAi molecules are not processed out of the VA1 transcript, they are not a substrate for Dicer. There is no teaching in the prior art that any molecules inserted into the VA1 transcript are processed out of the transcript.

In fact as discussed above, the prior art teaches exactly the opposite. Specifically, Doglio et al. teaches that the antisense oligonucleotide inserted into the VA1 transcript is functional because the antisense oligonucleotide appears "on the secondary structure as 'extrusions' relative to the principal axis of the VA RNA." Because of this structure, the antisense oligonucleotides "are accessible to sense RNAs." See column 4, lines 54-60 of Doglio et al. The ribozyme sequences are functional in the same manner as the antisense oligonucleotides. See column 5, lines 26-28 of Doglio et al. Thus, Doglio et al. teaches that the RNAi molecules inserted into the VA1 transcript, i.e., antisense oligonucleotides and ribozymes are not processed from the VA1 transcript. This teaching of Doglio et al. is confirmed by Rossi et al. ('087) and Cagnon et al. That is, Rossi et al. ('087) and Cagnon et al. teach that it was known that the ribozyme in the VA1 transcript was not processed out of the VA1 transcript. See, e.g., column 5, lines 28-33 of Rossi et al. ('087) which teaches that the ribozyme is active without being removed from the VA1 transcript. Thus, Applicants submit that the prior art clearly teaches that the RNAi molecules of antisense oligonucleotides and ribozymes are not processed out of the VA1 transcript into which they have been inserted by being expressed from an expression construct comprising the adenoviral VA1 gene and a nucleic acid encoding the antisense oligonucleotide or the ribozyme.

Since the prior art (Doglio et al., Rossi et al. ('087) and Cagnon et al.) teach that the antisense oligonucleotide RNAi molecule or the ribozyme RNAi molecule is not cleaved out of the VA1 transcript, Applicants submit that there is no motivation to substitute a different interfering molecule, e.g., shRNA or precursor miRNA which must be processed out of the VA1 transcript, for the antisense oligonucleotide or ribozyme of Doglio et al. which, as the art shows, is not processed out of the VA1 transcript. It is only upon removal of the shRNA or precursor miRNA from the VA1 transcript that it becomes a substrate for Dicer to produce the active siRNA or miRNA.

Furthermore, because Doglio et al., Rossi et al. ('087) and Cagnon et al. specifically teach that the antisense oligonucleotide RNAi molecule or ribozyme RNAi molecule are not processed out of the transcript, there is no reasonable expectation of success that any other RNAi molecule inserted into the VA1 transcript would be processed out of the VA1 transcript so that it could then function as a substrate for Dicer to produce the active molecule which would hybridize to the target mRNA. Since there is no reasonable expectation in the art that the shRNA molecule or precursor miRNA molecule would be processed out of the VA1 transcript to become a substrate for Dicer, Applicants submit that the claimed invention does more than yield predictable results.

These points are further substantiated by the Rule 132 Declaration of Dr. John Rossi as discussed in detail above.

In view of the above amendments and remarks, Applicants submit that the claimed subject matter is not rendered obvious by the combination of Agami et al., Doglio et al., either Yu et al. or Ambros and Lorens. Withdrawal of this rejection is requested.

**Rejection for Obviousness-type Double Patenting**

The Examiner has rejected claims 1, 2 and 11-16 under the judicially created doctrine of obviousness-type double patenting over claims 1 and 7-9 of Rossi et al. (US 6,995,258) taken with Agami et al. and Doglio et al. further in view of Zeng et al. (*Mol Cell* **9**:1327-33, 2002) and Yu et al. The Examiner concludes that that it would have been *prima facie* obvious to combine the teachings of these references to produce an expression cassette comprising an RNAi molecule operatively

linked to adenoviral VA1 promoter wherein the RNAi is shRNA or precursor miRNA. In addition to relying on these references to make this rejection, the Examiner also made the same comments with respect to motivation and processing as discussed above with respect to the obviousness rejections. Applicants traverse this rejection.

The Examiner cites Rossi et al. ('258) for its disclosure of an expression cassette that includes the U16 snoRNA and, for example, a hammerhead ribozyme specific to HIV-1 RNA and a TAR element. This construct results in the localization of the expression product to the nucleoli where it inhibits HIV replication. There is no disclosure in Rossi et al. of a nucleic acid encoding an RNAi molecule that is a substrate for Dicer that is inserted within the coding sequence of the adenoviral VA1 gene. Because the purpose of Rossi et al. ('258) was to achieve localization of the expression product in the nucleoli, there would be no motivation to modify the expression cassette in a manner that would lead to cellular localization and not nucleoli localization. There is no teaching in Agami et al. (which discloses subject matter as discussed above), Doglio et al. (which discloses subject matter as discussed above), Zeng et al. (which discloses miRNAs) or Yu et al. (which discloses subject matter as discussed above) that would provide any motivation for modifying Rossi et al. ('258) as proposed by the Examiner. Thus, the skilled artisan would not have combined the cited references in the manner proposed by the Examiner in making this rejection. Consequently, Applicants submit that the claimed subject matter is not obvious from the cited references. Since the claimed subject matter is not obvious from the cited references, Applicants submit that the instant obviousness-type double patenting rejection is improper.

As described above with respect to the obviousness rejections, Applicants submit that there is no teaching or suggestion in Agami et al. or Sharp et al. that the VA1 transcript is cleaved by Dicer in mammalian cells. In the absence of any such teaching in these references, the Examiner's assertions that a skilled artisan would expect that the RNAi molecules inserted into the VA1 transcript would be cleaved out to become a substrate for Dicer is incorrect, especially in view of the teachings of Doglio et al., Rossi et al. ('087) and Cagnon et al. as discussed above. As previously discussed, Applicants are not arguing that the shRNA or precursor miRNA molecules are not known

to be substrates of Dicer when they exit in the cell as such molecules. However, Applicants submit that these molecules are not known to be substrates of Dicer when they are incorporated into the VA1 transcript, i.e., VA1 RNA. If these RNAi molecules are not processed out of the VA1 transcript, they are not a substrate for Dicer. There is no teaching in the prior art that any molecules inserted into the VA1 transcript are processed out of the transcript.

In fact, as discussed above, the prior art teaches exactly the opposite. Specifically, Doglio et al. teaches that the antisense oligonucleotide inserted into the VA1 transcript is functional because the antisense oligonucleotide appears "on the secondary structure as 'extrusions' relative to the principal axis of the VA RNA." Because of this structure, the antisense oligonucleotides "are accessible to sense RNAs." See column 4, lines 54-60 of Doglio et al. The ribozyme sequences are functional in the same manner as the antisense oligonucleotides. See column 5, lines 26-28 of Doglio et al. Thus, Doglio et al. teaches that the RNAi molecules inserted into the VA1 transcript, i.e., antisense oligonucleotides and ribozymes are not processed from the VA1 transcript. This teaching of Doglio et al. is confirmed by Rossi et al. ('087) and Cagnon et al. That is, Rossi et al. ('087) and Cagnon et al. teach that it was known that the ribozyme in the VA1 transcript was not processed out of the VA1 transcript. See, e.g., column 5, lines 28-33 of Rossi et al. ('087) which teaches that the ribozyme is active without being removed from the VA1 transcript. Thus, Applicants submit that the prior art clearly teaches that the RNAi molecules of antisense oligonucleotides and ribozymes are not processed out of the VA1 transcript into which they have been inserted by being expressed from an expression construct comprising the adenoviral VA1 gene and a nucleic acid encoding the antisense oligonucleotide or the ribozyme.

Since the prior art (Doglio et al., Rossi et al. ('087) and Cagnon et al.) teach that the antisense oligonucleotide RNAi molecule or the ribozyme RNAi molecule is not cleaved out of the VA1 transcript, Applicants submit that there is no motivation to substitute a different interfering molecule, e.g., shRNA or precursor miRNA which must be processed out of the VA1 transcript, for the antisense oligonucleotide or ribozyme of Doglio et al. which, as the art shows, is not processed

out of the VA1 transcript. It is only upon removal of the shRNA or precursor miRNA from the VA1 transcript that it becomes a substrate for Dicer to produce the active siRNA or miRNA.

Furthermore, because Doglio et al., Rossi et al. ('087) and Cagnon et al. specifically teach that the antisense oligonucleotide RNAi molecule or ribozyme RNAi molecule are not processed out of the transcript, there is no reasonable expectation of success that any other RNAi molecule inserted into the VA1 transcript would be processed out of the VA1 transcript so that it could then function as a substrate for Dicer to produce the active molecule which would hybridize to the target mRNA. Since there is no reasonable expectation in the art that the shRNA molecule or precursor miRNA molecule would be processed out of the VA1 transcript to become a substrate for Dicer, Applicants submit that the claimed invention does more than yield predictable results.

These points are further substantiated by the Rule 132 Declaration of Dr. John Rossi as discussed in detail above.

In view of the above amendments and remarks, Applicants submit that the claimed subject matter is not properly subject to an obviousness-type double patenting rejection over Rossi et al. (US 6,995,258) taken with Agami et al. and Doglio et al. further in view of Zeng et al. and Yu et al. Withdrawal of this rejection is requested.

**Rejection for Obviousness-type Double Patenting**

The Examiner has rejected claims 1, 5 and 6 under the judicially created doctrine of obviousness-type double patenting over claims 1 and 7-9 of Rossi et al. (US 6,995,258) taken with Agami et al. and Doglio et al. further in view of Zeng et al. in further view of Lorens. In addition to relying on these references to make this rejection, the Examiner also made the same comments with respect to motivation and processing as discussed above with respect to the obviousness rejections. Applicants traverse this rejection for the same reasons as the previous obviousness-type double patenting rejection.

In view of the above amendments and remarks, Applicants submit that the claimed subject matter is not properly subject to an obviousness-type double patenting rejection over Rossi et al., Benn and Frey and Zeng et al. Withdrawal of this rejection is requested.

**Rejection for Obviousness-type Double Patenting**

The Examiner has rejected claims 1, 2 and 3 for obviousness-type double patenting over claims 1 and 7-9 of Rossi et al. (US 6,995,258) taken with Agami et al., Doglio et al. and Zeng et al. or Yu et al. in further view of Cagnon et al. In addition to relying on these references to make this rejection, the Examiner also made the same comments with respect to motivation and processing as discussed above with respect to the obviousness rejections. Applicants traverse this rejection for the same reasons as the previous obviousness-type double patenting rejection.

In view of the above amendments and remarks, Applicants submit that the claimed subject matter is not properly subject to an obviousness-type double patenting rejection over Rossi et al., Benn and Frey and Zeng et al. Withdrawal of this rejection is requested.

**Conclusion**

In view of the above amendments and remarks, Applicants believe that the present claims satisfy the provisions of the patent statutes and are patentable over the cited prior art. Reconsideration of the application and early notice of allowance are requested. The Examiner is invited to telephone the undersigned to expedite the prosecution of the application.

Respectfully submitted,

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